Chromatography

Components partition between two phases.

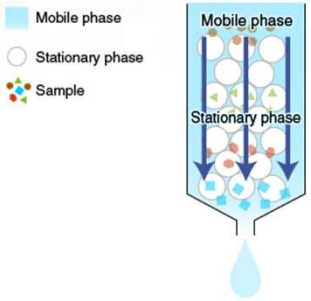
https://www.youtube.com/watch?v=0m8bWKHmRMM

1

1

Chromatography

The principle and method



http://ruo.mbl.co.jp/bio/e/support/method/chromatography.html

2

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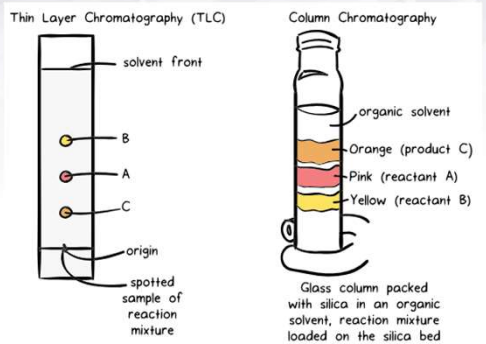
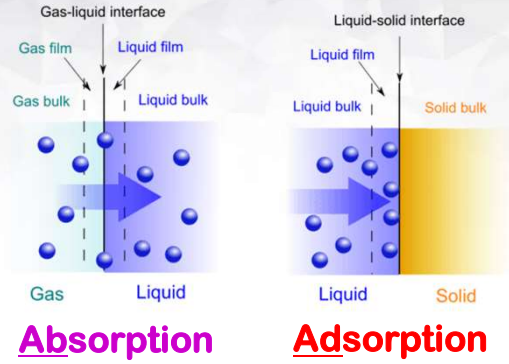


https://www.khanacademy.org/science/class-11-chemistry-india/xfbb6cb8fc2bd00c8:in-in-organic-chemistry-some-basic-principles-and-techniques/xfbb6cb8fc2bd00c8:in-in-methods-of-purification-of-organic-compounds/a/principles-of-chromatography

3

3



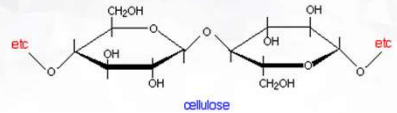
Absorption **Adsorption**

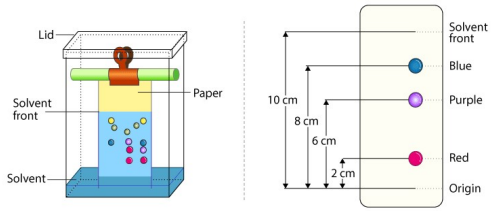
https://www.diffen.com/difference/Absorption_vs_Adsorption

4

4

Paper is made of cellulose fibres, and cellulose is a polymer of the simple sugar, glucose.





https://byjus.com/chemistry/paper-chromatography/

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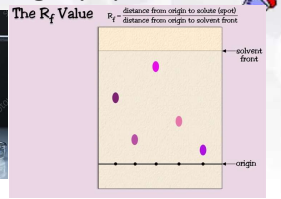
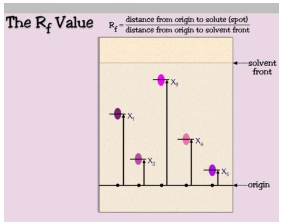
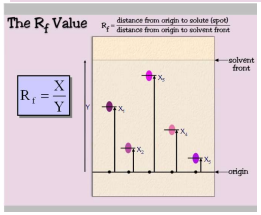
Paper chromatography





https://www.sciencephoto.com/media/860110/view/paper-chromatography



https://slideplayer.com/slide/4701883/

6

6

Table 12.5.1 . Properties of HPLC Mobile Phases		
mobile phase	polarity index (P')	UV cutoff (nm)
cyclohexane	0.04	210
n-hexane	0.1	210
carbon tetrachloride	1.6	265
i-propyl ether	2.4	220
toluene	2.4	286
diethyl ether	2.8	218
tetrahydrofuran	4.0	220
ethanol	4.3	210
ethyl acetate	4.4	255
dioxane	4.8	215
methanol	5.1	210
acetonitrile	5.8	190
water	10.2	—

https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Analytical_Chemistry_2.1_(Harvey)/12%3A_Chromatographic_and_Electrophoretic_Methods/12.05%3A_High-Performance_Liquid_Chromatography

7

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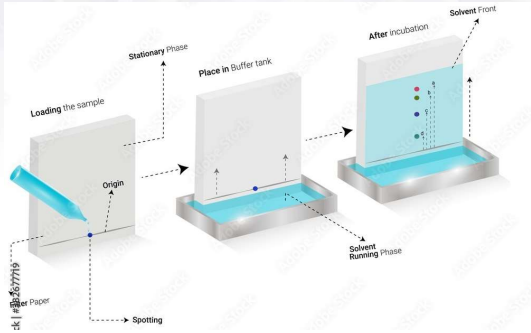
Ascending techniques

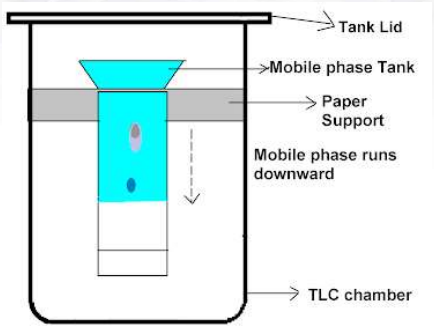


https://stock.adobe.com/th/images/ascending-chromatography-schematic-diagram-analysis-of-amino-acids-paper-chromatography-technique-biochemical-techniques/382677719

8

8

Descending techniques

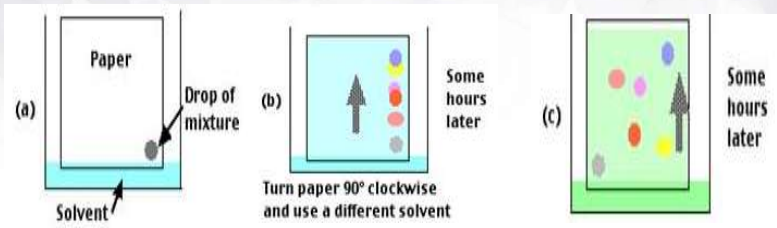


https://pharmainformationzone.blogspot.com/2020/04/descending-chromatography.html

9

9

Two-dimensional chromatography (2D)

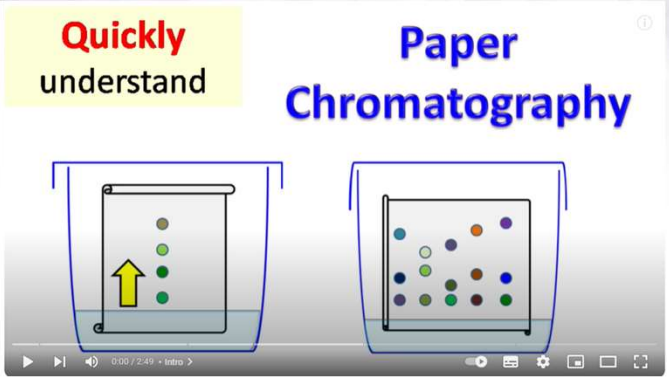


http://chromatographyscience.blogspot.com/2012/08/two-dimensional-techniques-in-paper.html

10

10

Quickly understand Paper Chromatography

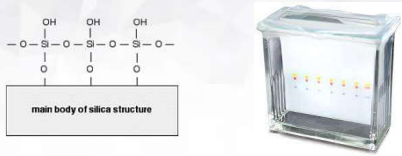


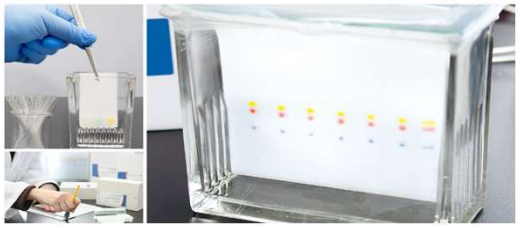
https://www.youtube.com/watch?v=mz_xcNrTK_U

11

11

Thin layer chromatography





https://mz-at.de/en/chromatography/further-categories/thin-layer-chromatography/
http://www.chemguide.co.uk/analysis/chromatography/thinlayer.html#top

https://www.linkedin.com/pulse/thin-layer-chromatography-tlc-market-size-evaluating

12

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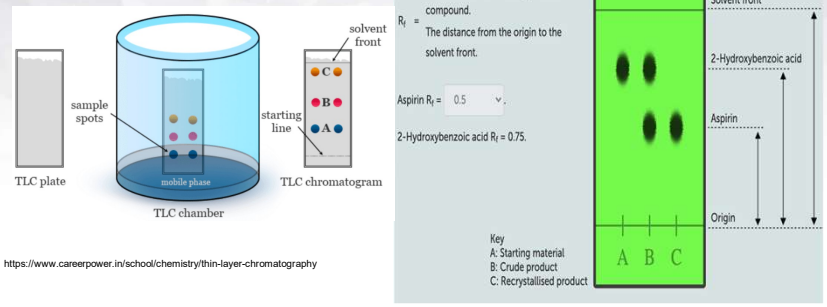


Using fluorescence

https://www.chegg.com/homework-help/questions-and-answers/see-two-spots-crude-product-thin-layer-chromatography-aspirin-q62594548

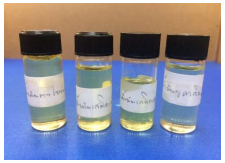
13

13

Comparison of different extraction methods: solvent extraction and steam distillation used for the analysis of the volatile components in *M. cajuputi leaves*

Mr. Suphachai Nampho

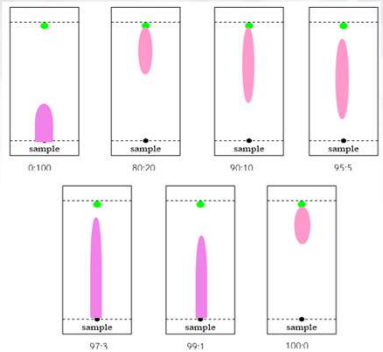
สารสกัดที่ได้จากการสกัดด้วยตัวทำละลาย



น้ำมันหอมระเหยจากการกลั่นด้วยไอน้ำ

14

14

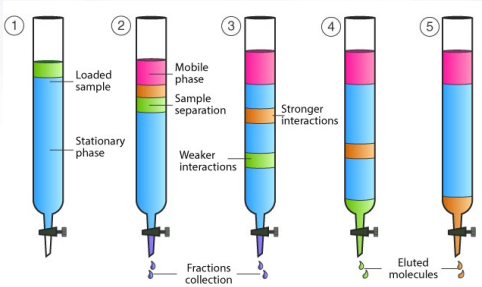


ผลการแยกสารสกัดตัวอย่างในอัตราส่วนเฟสเคลื่อนที่ต่าง ๆ (เฮกเซนต่อเมทานอล)

15

15

Separation of Plant Pigments by Column Chromatography (CC)

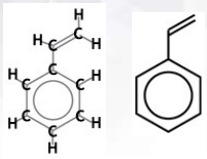


https://byjus.com/chemistry/column-chromatography/

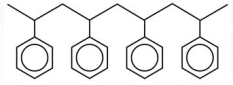
16

16

Ion Exchange Chromatography

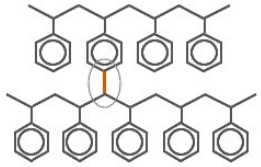


Chemical formula of styrene



A small fraction of a polystyrene chain

Divinylbenzene (DVB)



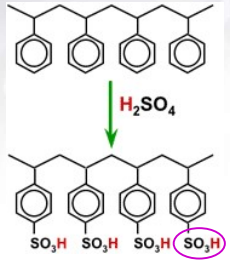
Cross-linked polystyrene

http://dardel.info/IX/resin_structure.html

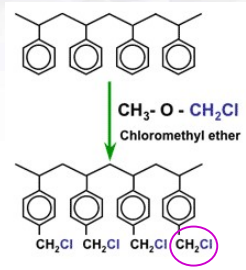
17

17

Resin Structure and Manufacture



- Cation Exchange
  - Sulfonic acid
  - SO_3^- (H^+)
  - Carboxylic Acid



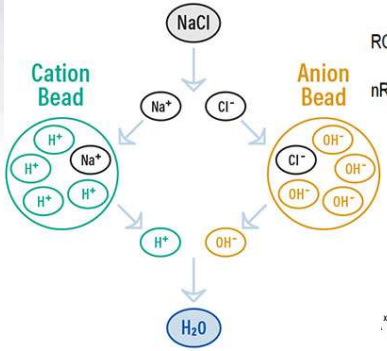
- Anion Exchange
  - Primary Amine
  - $NH_3^+(OH^-)$
  - Tertiary Amine

http://dardel.info/IX/resin_structure.html

18

18

Resin Structure and Manufacture



$$RCOOH + Na^+ + OH^- \rightarrow RCOO^-Na^+ + H_2O$$

$$nRCOO^-Na^+ + M^{n+} \rightarrow (RCOO^-)_nM^{n+} + nNa^+$$

$$xRCH_2N(CH_3)_3^+Cl^- + A^{x-} \rightleftharpoons [RCH_2N(CH_3)_3^+]_xA^{x-} + xCl^-$$

$$xRCH_2N(CH_3)_3^+OH^- + A^{x-} \rightleftharpoons [RCH_2N(CH_3)_3^+]_xA^{x-} + xOH^-$$

A^{x-} คือแอนไอออนต่าง ๆ เช่น OH^-, $SO_4^{=}$, NO_3^- ฯลฯ

19

19

Mobile Phase

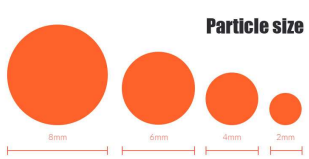
- Cation Exchange - H^+
- Anion Exchange - OH^-
- pH is controlled with a Buffer Solution
  - e.g. HCO_3^-/CO_3^{--}

20

20

ปัจจัยที่ส่งผลต่อเรซิน

1. size of particles



2. Degree of cross-linking

% Crosslinking	Moisture (%)	Capacity (meq/ml)
2	71	1.2
4	61	1.5
6	53	1.8
8	46	2.0
10	42	2.2
12	37	2.4
14	34	2.6
16	32	2.8

21

21

ปัจจัยที่ส่งผลต่อเรซิน

3. Strength of functional group

Resin	Functional group	Weak or Strong	Functional pH Range
DEAE	diethylaminoethyl [-N⁺(C₂H₅)₂H⁺]	weak anion	pH 2 - 9
ANX	diethylaminopropyl [-N⁺(C₂H₅)₂H⁺]	weak anion	pH 2 - 9
Q	quarternary amine [-N⁺(CH₃)₃]	strong anion	pH 1 - 14
CM	carboxymethyl [-O-CH₂COO⁻]	weak cation	pH 5 - 10
S	methyl sulfonate [O-CH₂-CHOH-CH₂-O-CH₂-CHOH-CH₂-SO₃⁻]	strong cation	pH 2 - 12
SP	sulphonyl group [-CH₂-CH₂-CH₂SO₃⁻]	strong cation	pH 2 - 14

4. Number of functional group

Nature	Functional group	Range	Applications	Capacity meq/g	Commercial resins
Strong acid	$-SO_3H$	pH < 2	Water softening	5	Lewatit S100, Amberlite IR-200
Weak acid	$=PO_2H$	pH > 4	Rare earth processing	10	Amberlite IFC-84 Duolite CC3
Strong base	$-NR_3X$	0 - 14	Uranium processing	4	Zerolit MPF Lewatit MP200
Weak base	$-NH_2$, $-NR_2$, -NHR	0 - 10	Uranium processing	5	Zerolite MPH Lewatit MP62
Chelating resin	Picolylamine $-N(CH_2C_5NH_4)_2$	-	Cu & Ni removal	1.5	XFS 4195

https://www.researchgate.net/figure/1-Classification-of-ion-exchange-resins-based-on-functional-groups_tbl1_232957574

22

22

Ion Exchange Chromatography – General Characteristics

- In general, ion exchangers favor binding of ions with:
  - ↑ charge
    - $3^+ > 2^+ > 1^+$; $Na^+ < Ca^{2+} < La^{3+} < Th^{4+}$
  - ↓ hydrated radius
    - $H^+ > Li^+ > Na^+ > K^+$
    - ↓ hydrated; exchange ↑
  - ↑ polarizability

Ion	Ionic Hydration Radius/Å
Li^+	3.82
Na^+	3.58
K^+	3.31
Rb^+	3.29
Cs^+	3.29
Ca^{2+}	4.12
Mg^{2+}	4.28
Sr^{2+}	4.12

https://www.researchgate.net/figure/The-hydration-radius-and-potential-of-the-ions_tbl1_370169111

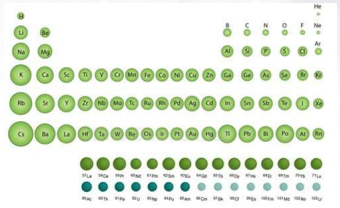
23

23

Separation of Cadmium and Zinc on an Anion-Exchange Resin, and Their Determination Using Standardized EDTA Solution

Metal ions	Ionic radius (Å)	Hydrated radius (Å)
Zn^{2+}	0.83	4.30
Cd^{2+}	1.03	4.26

$$Zn^{+2} + 4Cl^- \Leftrightarrow ZnCl_4^{-2}$$
$$Cd^{+2} + 4Cl^- \Leftrightarrow CdCl_4^{-2}$$



Sample Addition to Anion-Exchange Resin:

$$2R^+Cl^- + ZnCl_4^{-2} \Leftrightarrow (R^+)_2ZnCl_4^{-2} + 2Cl^-$$
$$2R^+Cl^- + CdCl_4^{-2} \Leftrightarrow (R^+)_2CdCl_4^{-2} + 2Cl^-$$

https://www.researchgate.net/figure/Radius-and-hydration-energy-for-Zn-2-and-Cd-2-18_tbl1_232987565

24

24

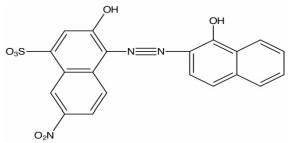
Elution of Zinc:

$$(R^+)_2ZnCl_4^{-2} + (R^+)_2CdCl_4^{-2} + 6OH^- \Leftrightarrow 2R^+OH^- + (R^+)_2CdCl_4^{-2} + Zn(OH)_4^{-2} + 4Cl^-$$

Elution of cadmium:

$$(R^+)_2CdCl_4^{-2} + 2NO_3^- \Leftrightarrow 2R^+NO_3^- + Cd^{+2} + 4Cl^-$$

Eriochrome black T (EBT) indicator



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25

The titration reaction between the zinc tetra amine complex and HY^{-3} (the form of EDTA predominating at pH10)

$$Zn(NH_3)_4^{+2} + HY^{-3} \rightarrow ZnY^{-2} + NH_4^+ + 3NH_3$$

26

26

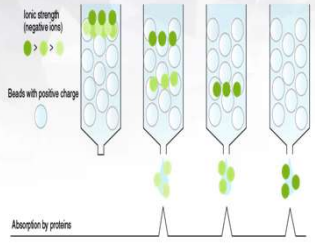
Ion Exchange Chromatography

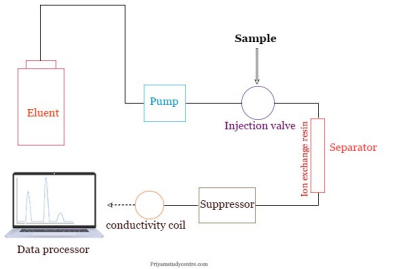
https://www.youtube.com/watch?v=i4U4ndf2ayg

27

27

Ion-exchange chromatography





Instrumentation of Ion exchange

http://ruo.mbl.co.jp/bio/e/support/method/chromatography.html

https://www.priyamstudycentre.com/2021/10/ion-exchange-chromatography.html

28

28

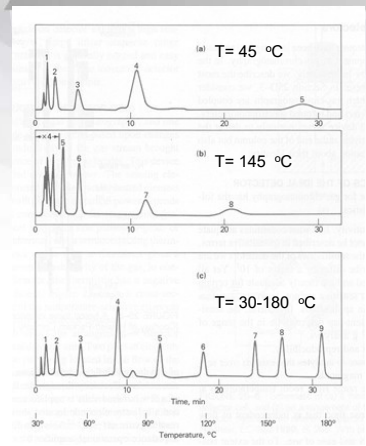
Gas Chromatography

https://www.youtube.com/watch?v=iX25exzwKhI

29

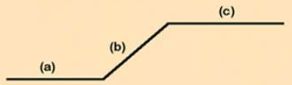
29

Isothermal & Temperature programming



https://slideplayer.com/slide/8198088/

A temperature program



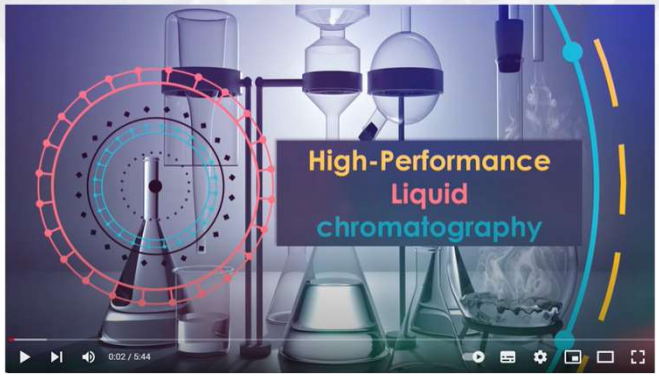
a - initial temperature and time
b - ramp (°C/min)
c - final hold time and temperature

Some GCs will allow for a more complex program.

30

30

High performance liquid chromatography



https://www.youtube.com/watch?v=eCj0cRtJvJg

31

31

HPLC

32

32

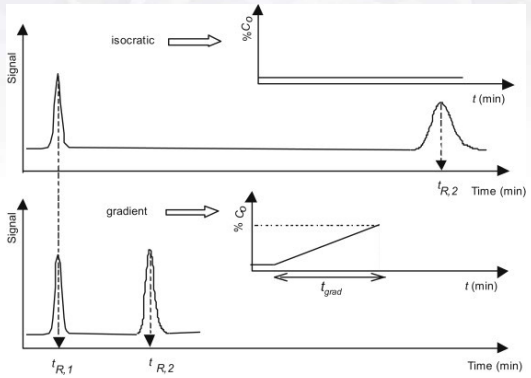
High performance liquid chromatography

	Normal Phase	Reversed Phase
Stationary phase	Polar (silica gel)	Non-polar (C18)
Mobile phase	Non-polar (organic solvents)	Polar (aqueous/organic)
Sample movement	Non-polar fastest	Polar fastest
Separation based on	Different polarities (functionality)	Different hydrocarbon content

33

33

Isocratic & Gradient elution

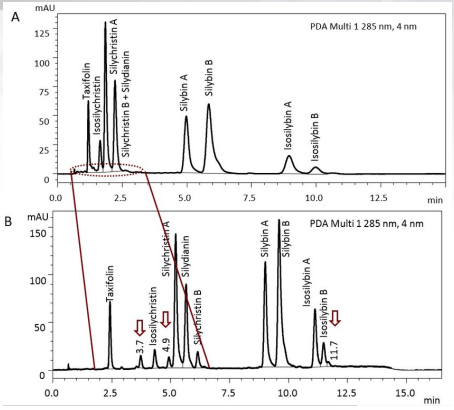


https://www.sciencedirect.com/topics/materials-science/gradient-elution

34

34

Chromolith RP-18e (100 × 3 mm) column; isocratic conditions: Mobile phase: 2% acetonitrile, 37% methanol, 0.1% formic acid, flow rate 1.1 mL/min, t = 25 °C;
gradient conditions: Mobile phase: A = 5% acetonitrile, 0.1% formic acid; B = 80% methanol, 0.1% formic acid; gradient: 0 min 30% B, 12 min 60% B, 13 min 60% B, 14 min 30% B, 16.5 min stop, flow rate 1.1 mL/min, t = 25 °C.

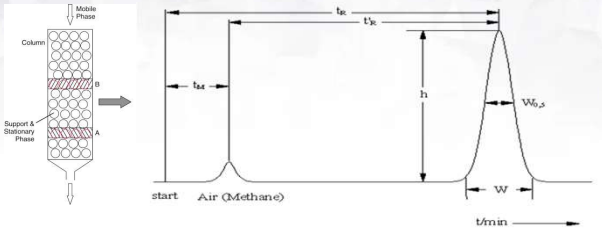


https://www.researchgate.net/figure/Comparison-of-the-isocratic-A-and-gradient-B-HPLC-analytical-method-for-silymarin-SM_fig2_338806376

35

35

The Chromatogram



t_M :retention time of air or mobile phase

t_R :retention time

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https://www.sciencedirect.com/topics/chemistry/chromatography

36

36

The Capacity Factor; k'

$$k'_A = \frac{K_A V_S}{V_M} \quad \text{.....(1)}$$

$$k'_A = \frac{t_R - t_M}{t_M} \quad \text{.....(2)}$$

k'_A : capacity factor

V_S : volume of stationary phase

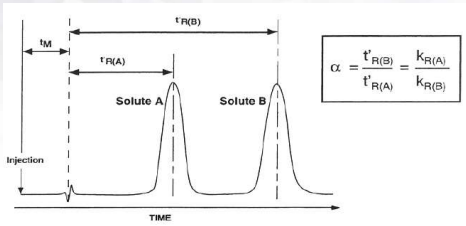
K_A : partition ratio

V_M : volume of mobile phase

37

37

The Selective Factor; α



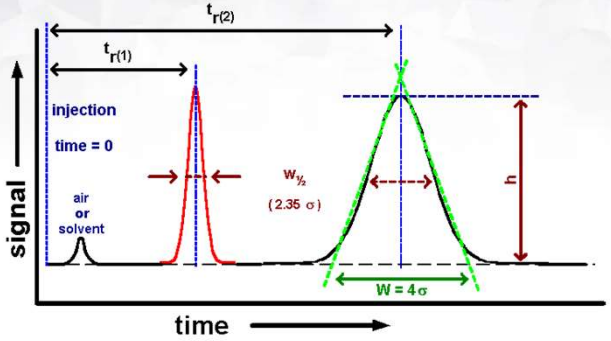
$$\alpha = \frac{K_B}{K_A} \quad \text{.....(3)}$$

$$\alpha = \frac{k'_B}{k'_A} \quad \text{.....(4)}$$

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} \quad \text{.....(5)}$$

38

38



39

39

The Efficiency of chromatographic columns

Number of theoretical plates; N

$$N = \frac{L}{H} \quad \text{.....(6)}$$

$$N = 16\left(\frac{t_R}{w}\right)^2 = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2 \quad \text{.....(7)}$$

L : the length of the column

H : plate height

40

40

Number of theoretical plates; N

$$N_{req} = 16R^2 \left(\frac{\alpha}{\alpha - 1}\right)^2 \left(\frac{1 + k_2'}{k_2'}\right)^2$$

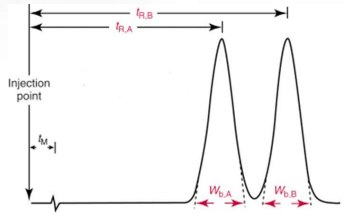
$$k_2' = \frac{t_{R_2} - t_M}{t_M}$$

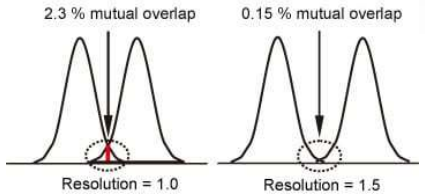
41

41

Resolution; R

$$R = \frac{t_{R_2} - t_{R_1}}{\frac{w_1}{2} + \frac{w_2}{2}} = \frac{2\left(t_{R_2} - t_{R_1}\right)}{w_1 + w_2} \quad \text{.....(8)}$$



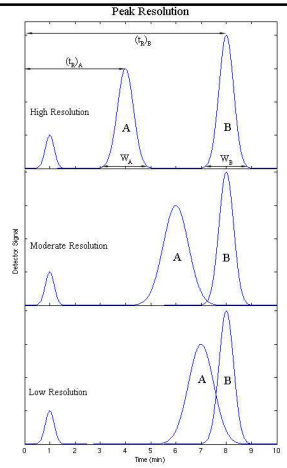


https://www.sciencedirect.com/topics/chemistry/chromatography

42

42

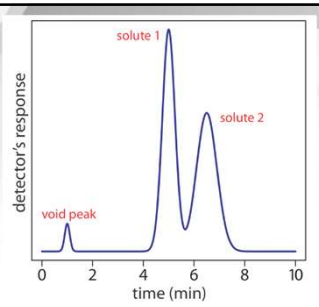
Resolution

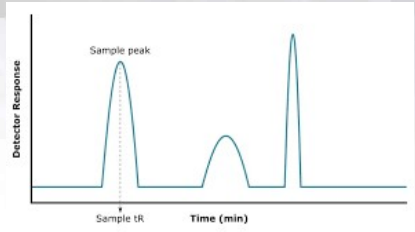


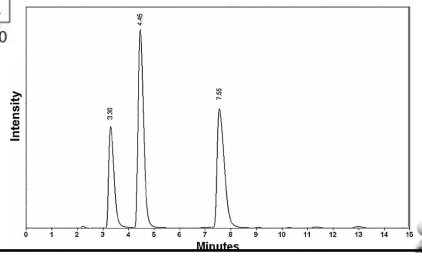
https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Supplemental_Modules_(Analytical_Chemistry)/Instrumental_Analysis/Chromatography/High_Performance_Liquid_Chromatography

43

43







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44

44

ตัวอย่างที่ 9.1

การวิเคราะห์วิตามิน B1 ด้วย HPLC โดยใช้สารมาตรฐานที่มีความเข้มข้น 5.00 mg/L ได้พื้นที่พีค (area) = 1200 หน่วย เมื่อฉีดสารตัวอย่างไม่ทราบความเข้มข้น ได้พื้นที่พีค = 1560 หน่วย ความเข้มข้นของวิตามิน B1 ในน้ำตัวอย่างเท่ากับกี่ mg/L (สมมติความสัมพันธ์เป็นเชิงเส้นตรง)

ตัวอย่างที่ 9.2

ในการวิเคราะห์คาเฟอีนด้วย HPLC:

- ความเข้มข้นของคาเฟอีนในสารละลายตัวอย่างที่เจือจางแล้ว = 3.50 mg/L
- ปริมาตรที่ใช้ฉีด = 20 µL
- ปริมาตรของสารละลายทั้งหมด (ก่อนเจือจาง) = 10.00 mL
- ตัวอย่างสกัดมาจากกาแฟ 1 ถ้วย (150 mL)

กาแฟถ้วยนี้มีคาเฟอีนทั้งหมดกี่มิลลิกรัม

45

45

ตัวอย่างที่ 9.3

ใน GC ใช้ **benzene** เป็น internal standard

- สารตัวอย่าง: โทลูอีน
- สารมาตรฐาน:
  - โทลูอีน 50 ppm → พื้นที่พีค = 1200
  - เบนซีน 50 ppm → พื้นที่พีค = 1000
- ในตัวอย่างไม่ทราบความเข้มข้น:
  - พื้นที่พีคโทลูอีน = 1500
  - พื้นที่พีคเบนซีน = 1200

ความเข้มข้นของโทลูอีนในตัวอย่างเท่ากับกี่ ppm

46

46

ตัวอย่างที่ 9.4

จากโครมาโทแกรมของ HPLC แสดงพีคของสาร 2 ตัวที่ตรวจพบดังนี้:

1. ค่า **k'** ของสาร A และ B
2. ค่า **selectivity (α)** ของ B เทียบกับ A
3. ค่า **resolution** ระหว่าง A กับ B

รายการ	สาร A	สาร B
Retention time (t_R)	3.2 min	4.5 min
ความกว้างพีคที่ฐาน (w_b)	0.40 min	0.50 min
เวลา dead time (t_0)	1.0 min	1.0 min

47

47

ตัวอย่างที่ 9.5

จากโครมาโทแกรมของการวิเคราะห์ด้วย GC พบว่า:

- สาร C มี t_R = 5.20 min
- ความกว้างของพีคที่ครึ่งความสูง ($w_{1/2}$) = 0.30 min
- ความยาวคอลัมน์ = 30 m

1. คำนวณ จำนวน theoretical plates (N) โดยใช้สูตรจาก $w_{1/2}$
2. คำนวณ plate height (H) ของคอลัมน์นี้

48

48

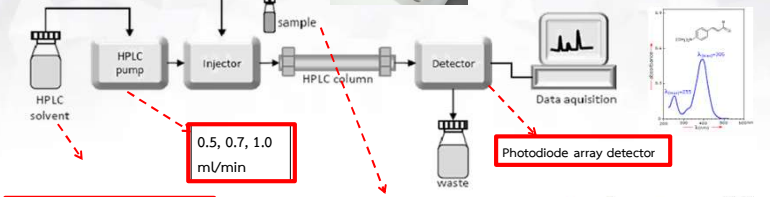
DETERMINATION OF WATER-SOLUBLE VITAMIN IN HONEY AND RICE BY HPLC

Saowaree Wongsirivitaya code 6004103346
Chemistry , Faculty of Science, Maejo University.

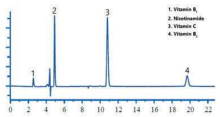
51

49

Scan λ_{max} อัตราการสแกนในช่วง 200-400 nm
เพื่อวิเคราะห์หาความยาวคลื่นที่เหมาะสม



Solvent A : 0.1% Fomic acid
solvent B : Acetonitrile
A:B = 75:25, 85:15, 95:5



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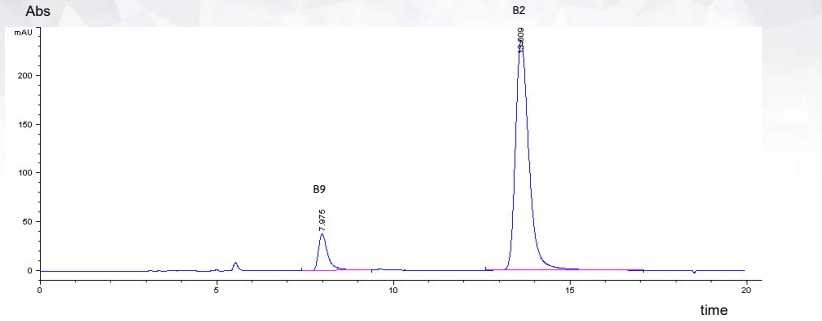
The optimize Condition of HPLC-DAD

Parameter	Condition
Mobile phase	0.1% Acetic Acid : Acetonitrile (85:15 v/v)
Flow rate	0.70 mL/min
Column	C18 (250 mm x 4.6 mm x5 µm)
Detection wavelength	267 nm
Retention time	20 min
R_t	B2 = 13.61 min B9 = 7.98 min

51

51



52

52